

Supplementary materials

Mass spectrometry of the >250 kDa protein purified with monoclonal antibody 8E3.

The >250 kDa band from the 8E3 immunoprecipitation was excised from the gel, destained, reduced with dithiothreitol, alkylated with acrylamide and digested with trypsin (Promega). The resulting peptide solution was analyzed on a Micromass CapLC and Q-Tof API US (Manchester, UK) LC-MS system. A peptide trap packed with Poros R2 (Perseptive Biosystems, Foster City, CA, USA) was used for online desalting, followed by separation on a 0.075 x 100 mm reverse phase column packed with Vydac C18 (Vydac, Hesperia, CA, USA). Peptides were eluted from the column with a linear gradient of 3-45% solvent B (solvent A: H₂O, 2% ACN, 0.1% formic acid; solvent B: ACN, 2% H₂O, 0.1% formic acid) at a flow rate of ~300 nL/min. The standard Micromass nanospray source with blunt tip 90 mm OD, 20 mm ID fused silica emitter was held at 80° C, capillary voltage +3.4 kV, cone voltage 32 V. Data acquisition was performed in data dependent mode, with up to 3 precursors for MS/MS selected from each MS survey scan. The .pkl files generated by Micromass ProteinLynx software were searched against the *Neospora* database

(<http://www.sanger.ac.uk/sequencing/Neospora/caninum/>) using the Mascot MS/MS Ion Search (<http://www.matrixscience.com>). Ten tryptic peptides were identified that matched a region of the translated genome. The resulting regions containing tryptic peptides were subjected to BLAST analysis of the *Toxoplasma* genome (<http://toxodb.org/ToxoDB.html>).

Table S1. MS/MS-identified peptides of *Neospora* 8E3 immunoprecipitated protein.

<u>Peptide Identified</u>	<u>Amino Acid Position</u>
KLVSSVPIDDLNAPGVDIGTFK	224-244
RLLDVTLPPVTIGGDLTR	291-306
RGENAPEFAYR	338-347
KLPTLFVLDDANDR	557-569
RMSDEDLQGVADYLIFR	830-845
RHLNIPVADVAMSEELNR	1076-1092
KFENVFNFSFEVMDK	1182-1195
RSLPEDGQVSFEYR	1520-1532
KSLIEGLQPTESTDSVLTAILR	1548-1568
KSLEEVALLLSK	2043-2054

Knockout constructs

For generation of a RON8 deletion vector, upstream DNA regions (amplified with forward primer AGTAGCGGCCGCTGGAAAGCAGTGTCGCAGTTG and reverse primer GCGCTCTAGAGCGATGCAAGACGATTCAAGC) and downstream DNA regions (amplified with forward primer CAATAAGCTTCATCCGGTGGTGTACGACATC and reverse primer GCCAGGTACCTCGCCTTCTACGGCATACTG) flanking the coding sequence encoding residues 1-1716 were amplified from *T. gondii* RHΔ*hpt* strain genomic DNA and subcloned into the pMini-GFP.ht vector (Arrizabalaga *et al.*, 2004). This vector contains the selectable marker hypoxanthine-xanthine-guanine phosphoribosyl

transferase (HPT) gene, and a GFP cassette located downstream for negative selection of heterologous recombinants. The 5' flank (~3.3 kb) was inserted using NotI and XbaI and the 3' flank (~3.3 kb) was inserted using HindIII and KpnI. The final construct (*RON8 KO*) was sequenced at the junctions, linearized by KpnI digestion, and 30 µg of DNA was transfected by electroporation in seven independent experiments into RHΔ*hpt* parasites. A construct which disrupts the *Toxoplasma* gene encoding PP2C-hn (Gilbert *et al.*, 2007) was used as a positive control. For selection of transformants, the transfected parasites were grown in media containing 50 µg/ml MPA and 50 µg/ml xanthine. No RON8 knockout parasites could be detected by PCR or IFA of the seven populations, whereas PP2C-hn knockouts were readily recovered in multiple trials carried out in parallel.

Toxoplasma and *Neospora* *RON8* sequence alignment

The *Toxoplasma* RON8 sequence agrees with gene model 541.m00141 and was confirmed by EST and cDNA sequencing. The *Neospora* RON8 sequence was determined *in silico* using the *Toxoplasma* RON8 sequence as a model and visual examination of intron/exon boundaries. The two sequences were aligned using Clustal W at <http://www.ch.embnet.org/software/ClustalW.html>. (*) = identity, (.) or (:) = similarity

TgRON8	MVATTLHSLPSRYLYTLLMSFLFVCGALALHESPDGLPEAPDRPQNAESGDGPPTLVLT
NcRON8	MVAATLRRSSRTRYLYTFLLATLFVCGDLALHKSANASSEAREDSDAVSDDGFTTLVLTV ***:***: * :*****: * : * ***** * * * : . . * : . * : * . * . ***:*****:
TgRON8	DPEYAGKSEEEAFTAEYFPPTESSKNEDGAQQSFVETPNRRLPGTFDPMSSSDVRSEVQL
NcRON8	DPEDSGKSEEEAFTAEYFPWEASKNDD-VKQSFFEAPNRGLPGKFDPMSPSDVRSVQL *** :*****:*****: * :***: * . :*** . * :*** * . ,****.*****:***
TgRON8	RVTTQQMERGLLAETIVVLQEGQMVGFRASLQTKEQAALLHRLADLTNDPNIHFRVEWRG
NcRON8	RVTTQQTERGLHAEVIVLQEGQMISGFRASLQTKEQAALLQRLADLTSDPNIHFRVEWRG ***** *** * :*****:*****:*****:*****.*****

TgRON8	RGDGTGGLYIRTAVHLLATAYKPSVHFKLNHLQTGARGARETIKLLSSVPIDDLNAPGVD
NcRON8	RGDGSFSVIIRTAVHLLATAYKPSVHFNLNLHQIGPGVRQTIKLVSSVPIDDLNAPGVD *****:*. : ****:*****:*****:***** * . *. :*****:*****:*****
TgRON8	IGTLKQLIVDHAEPENILTGPGSFSIKRMFKESDPKNANTQEATLVPWLLRLLDLAPVTD
NcRON8	IGTFKQLISDHAEPENIITGPGSFSIKRMYNESDPKNVNTQEATLVPWLLRLDVLPVT ***:***:***:*****:*****:*****:*****.*****:*****:***
TgRON8	IVGDLTRNREFSTVLPQGPANWNDLILYTMGDAANRGQNAPDFAQREPGYRPGSIFLS
NcRON8	IGGDLTRNREFSTVLPQGPANWNDLILYTMGDAANRGENAPEFAYREPGYRGGSFLS * *****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	PDGFRGAALGHDQPDRLKQFWRQGETGLNPGDIEHYYSPLFGRSYDTSNNPLLLDMY
NcRON8	PEGLRGAARLGDHQPDRLKEYWRQGETGLNPGDIEHYYGPLFGRTYDTSNNPLLLDMH *:***:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	GNPVYSEGGVPKFIVGPGENDLTKLPDHVSGMVLPSVSARFLFPDKRSFTEFINPARPWN
NcRON8	GNPLYSPNGVPRFTVGPQTDLSKLPDHVSGMVLPSVQTRFAFPGNRGFSEYINPARPWS ***:***:***:***:*****:***:*****:*****:***:***:***:*****:*****
TgRON8	DISKGWKALMAHKMKFNREPLGLVGVSVNGNWYGLTSEFIQTYPTLLHLLGSLAQSTPG
NcRON8	DINNGWKALMAHKMKFDREPGLVGVSVNGNWYGLTGFVENYPTLLHLLGSLAQSTPG **.:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	FSLEDVTLFHDDKLPPKLPTLFLVLDAAHDRQIPWCAFEVPIPLSPRGTVNIKSLLAKGKS
NcRON8	FSVEDLTFFHDDKLPAKLPTLFLVLDANDRQWPWCafeVVPIARGTVNIKSIMAKGKA ***:***:***:*****:*****:*****:***:*****:***:*****:*****:*****:*****
TgRON8	AAESPKGCNFREKGHFHTTVHRWGFVESRNPLSIQWHGVLDYTGSKQECALLGNVLKDAH
NcRON8	AAESPKGCNFRERGFHFHTTVHRWGYVESRTPLKIEWHGILDYHGSKEECSLLGKVLKDAH *****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	LAGRQIAVSFHAEHKATVIPVIETFGPDGELLKSKPQKPVTATVQFGDDLPGLLYDFHKP
NcRON8	LARQIAVSFHAEHKATVIPVLETFGPDGQRLTAKPQKPVTATVQFGDDLPGILFDFHKP ** *****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	FQKKKVPTVVYPFLQGQGQS--RDALNGGVLGEEYYHPRKTKITMRPEIPILNTHDTF
NcRON8	FQKKKVPTVVYPFLPGRGQGGGPQDALNQGVLGEDYYHRPQKTRIMMRPEIPILNNAHDTF *****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	VDGSGPHPNVPHFVIPTAGPQQHLAPLKALRQWLRKEYPGSQLPKLDRMSDEDLLGIA
NcRON8	VDGSGSHPNVPHFVIPITTGQQHLAPLKAFRHWLRTAYPGSQLPFNLDLDRMSDEDLQGVA *****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	DHLVFRFPDGTEATLRSIFGPEVFSNPWATMNPDAYQKLMVDLVRPKVYLAKNFRIVVT
NcRON8	DYLIFRFPNGREASLRAIFGPELFSNPWATVNPEAYRNLMQLIAQPKKVYLAKNFRIVVT *:***:***:***:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	DVDGQQIPFDIQIEPGDTWAKTLDDFFKAHPNLK PANVKLVLYDDKDTALRQFDINLD MY
NcRON8	DADGROIPFDIQIEPGDTWAKTLDAFLKAHTPIK PANIKLVLYDDKDSALRQFDINLD MY *.*:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	ASPYTEAVDQOHMKGLQITLETPAPIVSCYQADPARPGOCLAVDMKRIFCGRVNPAKMVS
NcRON8	SSPYTEAVDQOHMKGLQITLETPAPIVSCYQTDPARPGOCLAVDMKRIFCGRVSPAMLS :*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	RAWNEECTAAWIARADLGEVEDRYLMGPDDVRARSIRDLITSAKNALQQRNPEKAKHL NI
NcRON8	RAWNDECTAAWIAHADLGEIEDRYLMGQDDVRARSIRDLITSAKNALQKHPERVRHL NI *****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	PVADVTESEELNRKMRERTELAMKSGKTLEDDRLRHLKFEIRTLOROGELOWAKLVGAT
NcRON8	PVADVAMSEELNRKMRERAELTVKSGKTLEELDRLRQLKFEIRSLQROGELOWARLTGAT *****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****

TgRON8	ASEGEGLDFP-GVFRGGTLGDMMKGMNLSPQTTYGDFVRTVKKKINSPEGLTIPGVRQHVG
NcRON8	AKEREGAFPGVVFRCGSNLNDLLKGMHMSPQTSYGDVRTVTKNSPGGLEVPGIPQHIG *.* ** * ****:*. :***:***:*****:*****. :***** * :*** :***
TgRON8	GSGRPAQQIIDFDFAFPESPPGAAGKPGWEATWRPATVGAFPDNMPIGDIDREMHHFLV
NcRON8	GTTRPGQSAVIDFDAAPDSPASGGQPGWSASWRPATVGAFPDNMPIGEIEKDHLHHPFLV *:***. : .. .*. :***. :*****. :*****:*****:*****:*****
TgRON8	FTTG-HPPKYDLNGLAAYGGQPFREETTHLDASLRNLVEWMKDSLGIIDPNGLAEIVID
NcRON8	FVTGGRAPKDYGIASYGGRPYHEEMKLDASLRNLVEWMKDSMGIPDPNGILADVVID *.* :**** * :***:***:*** .*****:*****:*****:*****:*****
TgRON8	TIRCPFPLSAAQLISATIGHLLHHCQVNDEMSHSIYVTPTPVPGYTATPKRNGPO-IVI
NcRON8	TIRCPLPLTAAQLFSLTVGHLHHCHVTDLETSHSIYVTPTPVAGFTAAPKRNGPQQIVI *****:***:***:***:***:***:***:***:*****:*****:*****:*****
TgRON8	NGHNYGAPGTAPRDFGQLFNLPGRPGARPLNQDLEVSFSPLPGKPPVRGVLPDVPLLQ
NcRON8	NGHKYGAPGTAPRDFAQFLNLPGTPGTRPLKQDLEISFSVPGKPPVRGVLPDVPLLQ ***:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	SISDKHGQPGVAVVLFRLRQLHALAELPPGATLNIDTKLIPSQSPOQLYAPDQVQPQGPW
NcRON8	SISDKHGQPGVTVVLFRLRQLHALAELPPGADFNIDTKLIPSQSPOQLYLPGEVQPQGPW *****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	IAGPTGGGRFPGGGVVVNESGRRGPGGSAAVKFMPSTLRLPPLLQPSVELPRNSMLRGS
NcRON8	LTGPVG-----PGSRGVVNLLPSGLRPLLQPSVQLPRNSMLRGS *:***. * ***. :***:***:*****:*****:*****:*****
TgRON8	IGGVGGLPRTEFVVPILPSQKSMPIEQVLKFFGIAPAAVQTLDVQEKEQGAILIESGTV
NcRON8	IGGVGGLPRTEFVVPILPSQKSMPIEQVLKYFGIAPAAVQTLDVQEKEPEGVITVSGKV *****:*****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	TFVPLKGPTFTVSMKKINATDKNTLATLLKAAGRSIIDLVGASDGTQPLVLKIVVDHGAV
NcRON8	TFVPLQGPAFSISMSKINSTDNNSLAALLKAAGRSVADLIGAAASTQPLVLKIIIDHGAT *****:***:***:***:***:***:***:*****:***:***:*****:*****
TgRON8	SPTKVTPVGTPSHARSAMEGLTIWNLLPPLIDVHSVKITIRVQAAKAFQQAAKAAAAA
NcRON8	SPTKVTPVGTPSHAKSALEGLTWKILPPLIKVHSVQTVTIKVQAKEFQTAKAAAAA *****:*****:*****:*****:*****:*****:*****:*****:*****:*****
TgRON8	EGTEGDSAGEPLSRPKNVEPAVRTKFKNKIPELPAAFKPRQRTVSEINGAPNPKLVIFVE
NcRON8	EGAEAGAAGESATRP-KAAPEASTKFSNKLPNLPAAFVPRQRDAEIEGGPDPNLLIFL *:***. :***. :** : . * . ***. :***:*****:*****:*****:*****:*****:*****
TgRON8	QDGQKTIRFTFPSRVNDVVGQSTVSVVLDAALLPNPGTWHRTFWGALSPDGTCKPTMEF
NcRON8	QNGQTTIKFNLRSRINDAVIGQSTVSRSRMLDVLPQPNSHRIFWGLQT-DGRACRPTLEF *:***. :***. :***:***:*****:***:***:***:***:***:***:***:***:***
TgRON8	QYPHWLQTKMAELPVNPNHSSCLLIREVSLNIKQRPNFFIKPYIFHSPLVIFALQGKQRI
NcRON8	AYQHWLQTVSALPVNPQSTCLLIREVPLNIKQPPTFPKPYIFHSPIVILALQGQKQTS * *****:***:*****:***:*****:*****:***:***:*****:*****:*****:*****
TgRON8	IFEEQVDPAVTSQPLTALLEQLGIPKTQLDTIQCSTNIDAPGECPSDSSTTVDPETVI
NcRON8	IYEEQVDPAVTSQPVTVLLKQLGIPTDQLGTVLQCSSTMIDAPGECPSSVTVDPETTV *:*****:*****:***:***:*****:***:*****:*****:***:*****:***:*****
TgRON8	PDAVATQSAFLVVTGKQKQAPGSFLQVLWRRQRRRNSIQKRITSH---
NcRON8	LDTVATKSAFLIVNGK--EASGSFLQVLWRRQRLNAVRKRKASQEYI *:***:***:***:***:***:*****:*****:*****:*****:*****:*****

RON5 protein sequence:

The entire RON5 coding region was determined by EST and cDNA sequencing, and was used to generate the protein sequence below.

MAEFTWRPLLMSLPKMIAFFHILLFSGALAAAAGSPAADLVASVQTVSNERKDL
YARDTQPTARTGIDIGVSFTQQASGNARTFEIRQHGSGPPRPAPRRAAAVADDIF
GSEDFSPPMNVAGAPLRDMGVHFLECQATDGKIECTGQGAGARPPFFRGGVDP
TEIHEIVQSRTVGPADYDEERPEQTDPYLSPVTLQRFVSSANASNSPLLEDPV
QVCLSRRKPTYTCHLLHEFAATSVIVEESGNLVCEDKAPLTVAEKRKINDAVKA
GRTPQATGGQSSRPPNPTVPSKAGAAPQNAASRQPVSFVEQENSEASMPTANTE
QASATTEDTKIASAATDSDGYGEAAAGESAQEGDRPPPYNPDADEAGVPRAVQE
AYEEARPLQEATIDKFQDAAAAAEEAADHFAQVSFVNAMQSALTISAGYHLR
AGSHVVLSACKRLVEAVAANPPGPGTVIPLEELRMQLVATLTQDFALAQAFIGDY
AIHVHSIAETLTPQMVAANALLESGIEELINHTARVKSRLAARGQDSPANVRKEI
VQESFRQLKVELFQEIVTRVCELMDDPESFLKTVPIIVGTTPTAPLRTGGHLGADY
IIHLRNNDLCDVTASDAQIFPSAPADEFGLQGFPRHNLGERLVGWMMDVMARTKTAR
KEVFKIIDFTKAKDVLLFASETWKARYATLQAPAAPAPSFYGITSGCRNVNDIM
KSKFFDMYLMHQSGFMKLQRHGNDSRQRFMHRVTLQNDGILPQLPLEADYEL
MELNAAMQKNFVAANKSIFSRRARHSKYGYLDLCDVACYQKIDRLHNDVMT
NVFFSLDTLMKIVAKVHRSYGIAKAFFQLGARQHHIADPNLGWARRLFVHW
ASHNEVKQMKGKVVKVNYENLRHGEFTLDTVRMRDALVRYTNMLKADPITR
DLMSLVIAHTWIHIRGVRNAAMGFKNSQKLNESMNASAIGAVFAKLWYESDISVV
APHQELKPFGAPLASMALQIGFFLHTVVEYKMSLLEKAGAQIKSWFVGMFQKN
KRRTVPRTWKAVVAATNRAPKVNLCAYQGALLVIKTLAKMFRERFLYRFYM**Q**
GQGSKVDFNTPTLLIHALVASWMDPSLDRLELSSRTIPNAKKLFWYYVVWNENG
PSNAATRIVLTGCKKYTFLLPGVVRVSSTSSEVVEAGSNILKIDKIILKRSSLEAY
MNHLQATYDDPLTIVQVALDLAARCEGYSAAKDQPAQAMRGPARVRRATGEST
TFTIRGGVQGGTMSFVEAEADDERKEDSEDNTVDLSEQDQDSSFVQLKKLFNR
RGSSAAGQAVQTDAQPLPKAVQTDAQPLPKVRRGGPDVDASAVILGSRFMLDL
WCSKYRKMLVEKLSGISTKDATVMQQEISKVFSAVSSIKIAIPDYKDLWDFSLRC
DWMDGYPDAEKMRAARAEMVTYAMAKASTGKRLKMLQKVRSWIRKKFA
AARKLKSLKNRISTAAGRGRGKPPKAKVPDWAVVNAGMGMWTGKFSTHLTNE
DEMSCNGPHEPIRVMSWKQNHFTFASSSTNAERNYVLVKKGDDSHCWATREA
LVHKGWSGIPVYQYAEPAFWLQEVSNSQPFVVWDGYLTTSDNLTLQDIDIN
ASSDSLKSHAVMRIIVDSNGKTIYQGPPTGVVQTQGGVVTLSIRNLVSGVHSTG
DSVEVRTASGPQLTSVALDTQFKEIPDLVLR